

## Former Diversity of *Ephedra* (Gnetales): Evidence from Early Cretaceous Seeds from Portugal and North America

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• **Background and Aims** The extant species of the seed plant group Gnetales (*Ephedra*, *Gnetum* and *Welwitschia*) have been considered a remnant of a much greater, now extinct, diversity due to the pronounced differences in form and ecology among the genera. Until recently, this hypothesis has not been supported by evidence from the fossil record. This paper adds to the expanding information on Gnetales from the Early Cretaceous and describes coalified seeds from Barremian-Albian localities in Portugal and USA.

• **Methods** The fossils were extracted from sediment samples by sieving in water. Adhering mineral matrix was removed by chemical treatment. Seeds were investigated using light and scanning electron microscopy. Morphology and anatomy of the seeds were documented and compared with those of extant species.

• **Key Results** The fossils share characters with extant *Ephedra*, for example papillae on the inner surface of the seed envelope and *in situ* polylicate pollen grains that shed the exine during germination. They differ from extant *Ephedra* seeds in morphological and anatomical details as well as in their smaller size. Two new species of *Ephedra* are described together with one species assigned to a new genus of Gnetales. Other *Ephedra*-like seeds, for which pollen and critical morphological details are currently unknown, are also present in the samples.

• **Conclusions** These Cretaceous seeds document that key reproductive characters and pollen germination processes have remained unchanged within *Ephedra* for about 120 million years or more. There is sufficient variety in details of morphology to suggest that a diversity of *Ephedra* and *Ephedra*-like species were present in the Early Cretaceous flora. Their presence in Portugal and eastern North America indicates that they were widespread on the Laurasian continent. The fossil seeds are similar to seeds of Erdtmanithecales and this supports the previously suggested relationship between Erdtmanithecales and Gnetales.

**Key words:** Early Cretaceous, *Ephedra*, *Erdtmanispermum*, Erdtmanithecales, *Eucommiidites*, fossil pollen, fossils, Gnetales, Portugal, Potomac Group, seeds.

### INTRODUCTION

The three extant genera *Ephedra*, *Gnetum* and *Welwitschia* comprise the Gnetales, a small group of seed plants with a poorly known evolutionary history. The three genera are very different in overall form and ecology and the monophyly of the group has sometimes been questioned (Eames, 1952; Nixon *et al.*, 1994). However, several molecular studies (Goremykin *et al.*, 1996; Bowe *et al.*, 2000; Chaw *et al.*, 2000; Magallón and Sanderson, 2002; Rydin and Källersjö, 2002; Rydin *et al.*, 2002) support monophyly of the Gnetales, as well as the position of *Ephedra* as sister group to a *Gnetum*-*Welwitschia* clade. Gnetalean monophyly and this pattern of relationships was hypothesized originally based on morphology (Crane, 1985; Doyle and Donoghue, 1986, 1992; Doyle, 1996).

Arber and Parkin (1908) interpreted the marked morphological differences among the three extant genera as an indication of a long evolutionary history and, even though no unambiguous fossil representatives of the group were known at that time, they suggested that extant Gnetales constitute merely a remnant of 'former greatness' (Arber and Parkin, 1908). Later studies of dispersed pollen lent support to this suggestion by documenting the extensive

fossil record of polylicate pollen grains often referred to as 'ephedroids' that in general morphology are very similar to the pollen of extant *Ephedra* and *Welwitschia* (Wilson, 1962; Crane and Lidgard, 1989; Osborn *et al.*, 1993; Crane, 1996; Osborn, 2000). Discoveries of well-preserved and informative mega- and mesofossils from the Early Cretaceous have provided further undisputed support for a long and rich fossil history of the group. *Cratonia cotyledon* (Rydin *et al.*, 2003) and *Welwitschiostrobus murili* (Dilcher *et al.*, 2005) from Brazil, and possibly also *Drewria potomacensis* (Crane and Upchurch, 1987) from Virginia, USA, are Early Cretaceous members of the *Gnetum*-*Welwitschia* clade. Otherwise many of the Early Cretaceous gnetalean fossils in the literature appear more closely related to extant *Ephedra* (Wu *et al.*, 1986; Cao *et al.*, 1998; Krassilov *et al.*, 1998; Guo and Wu, 2000; Wu *et al.*, 2000; Sun *et al.*, 2001; Tao and Yang, 2003; Rydin *et al.*, 2004; Yang *et al.*, 2005). There are also Mesozoic fossils, with affinity to the Gnetales, which are difficult to assign to any of the extant lineages (Krassilov, 1982, 1986; Krassilov and Bugdaeva, 1988; Duan, 1998; Sun *et al.*, 2001).

Extant *Ephedra* comprises approx. 35–40 species (Kubitzki, 1990) inhabiting arid environments of the northern hemisphere and South America. They are shrubs

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with opposite to whorled branching and reduced leaves that are decussate or in whorls. Female cones have fleshy or dry bracts and seeds with an integument surrounded by an outer envelope. The apical part of the integument forms a micropylar tube that extends beyond the outer seed envelope and serves as the pollen-receiving area. Male cones consist of microsporangioophores with fused microsporangia, surrounded by paired bracts. The pollen grains are distinctive with aligned ridges and valleys (polyplicate) that form alternating thicker and thinner regions of the exine (Steeves and Barghoorn, 1959; El-Ghazaly and Rowley, 1997; Osborn, 2000). The exine is discarded during germination (El-Ghazaly *et al.*, 1998).

All extant species of *Ephedra* are very similar in gross morphology and have few informative characters in gene regions investigated so far [18S, 26S, ITS, *rbcL*, *trnL*, *rps4* (Rydin *et al.*, 2004); *rps4*, ITS1 (Ickert-Bond and Wojciechowski, 2004); *rbcL*, *matK*, ITS1 (Huang *et al.*, 2005)]. Phylogenetic analyses indicate that major groups of species within *Ephedra* correspond to geographic regions, but relationships among these groups are not well supported (Ickert-Bond and Wojciechowski, 2004; Rydin *et al.*, 2004; Huang *et al.*, 2005). Within the genus, the absence of obvious morphological synapomorphies for extant subgroups, and the apparently homoplasious nature of many characters, have hampered traditional classification as well as phylogenetic reconstruction of relationships and molecular dating analyses. Groups defined on morphological characters (Stapf, 1889; Mussayev, 1978; Freitag and Maier-Stolte, 1994) are not fully consistent with the phylogenetic history of the genus indicated by molecular data. Huang and Price (2003) suggested that extant species of *Ephedra* evolved recently (Oligocene to Miocene) based on the paucity of base substitutions in the *rbcL* gene and an assumption of a molecular clock calibrated by landmark events. The current lack of morphologically well-defined subgroups impedes a placement of the fossils in any of the subgroups and thus also makes it difficult to date subgroups by fossil calibration.

In this paper a set of fossils are described from the Early Cretaceous Buarcos and Torres Vedras localities, Portugal and from Drewry's Bluff, Virginia, USA. The material consists of coalified *Ephedra*-like seeds, which based on differences in detailed morphology are assigned to several new species. The fossil seeds add to knowledge of the evolutionary history of the Gnetales and provide clear evidence for the early occurrence of several unique characters of extant *Ephedra*.

## MATERIALS AND METHODS

The fossils were collected from Early Cretaceous localities in Portugal and USA. Samples from Portugal were collected from fluvial sands and clays at two localities: Buarcos and Torres Vedras, in the western Portuguese (Lusitanian) Basin. Both localities have now been obliterated by town development.

The Buarcos locality was a partly overgrown road cut in the town of Buarcos, situated north of Figueira da Foz

(Beira Litoral region), 40°09'54"N; 8°52'11"W, Carta Geológica de Portugal 19C Figueira da Foz (Rocha *et al.*, 1981). Samples were collected by E. M. Friis and K. R. Pedersen in 1992–1997. A small palynoflora was reported from the Buarcos locality by Pais and Reyre (1981). Subsequently, rich mesofossil assemblages have been extracted from the sediment sequence including many angiosperms (Friis *et al.*, 1997, 1999, 2000a, b). The plant-bearing sequence from Buarcos is included in the lowermost member (Calvaria Member) of the Figueira da Foz Formation, thought to be of late Aptian to early Albian age (Dinis, 2001) or early Albian (Heimhofer *et al.*, 2005).

The Torres Vedras locality was an open-cast clay pit on the north-eastern outskirts of Torres Vedras (Estremadura region), about 1 km north-east of Forte de Forca on the road to Sarge, 39°06'13"N; 9°14'47"W, Carta Geológica de Portugal Torres Vedras 30C, (Zbyszewski *et al.*, 1955). The sample studied here (Torres Vedras 43) was collected in 1989 by E. M. Friis and K. R. Pedersen, in a sandy, lignitic horizon referred to the basal member of the Almargem Formation, at the same level as sample 44 described in Friis *et al.* (2004). According to Rey (1993), this part of the sequence is of late Barremian to early Aptian age. A late Barremian to early Aptian age of the Torres Vedras samples is also supported by mesofossil floras and *in situ* pollen grains (E. M. Friis, K. R. Pedersen and P. R. Crane, unpubl. res.).

Samples from the eastern USA were collected by P. R. Crane, A. N. Drinnan, E. M. Friis and K. R. Pedersen in 1988, from the Potomac Group sequence at the Drewry's Bluff locality along the James River, south-east of Richmond, Chesterfield County, VA (37°25'30"N; 77°25'15"W). All specimens examined here are from reworked clayballs sampled in the lower part of the exposed fluvial sequence, about 1–2 m above river level (Drewry's Bluff samples 163, 164 and 198). The Early Cretaceous sequence at the Drewry's Bluff locality belongs to the Patuxent Formation and the sediments are referred by Doyle and Hickey (1976) to the upper pollen Zone I of Brenner (1963), which according to Doyle (1992) may be of middle Aptian age. The clayballs are older than the sediments in which they occur and are referred by Brenner (1963) to the lower part of pollen Zone I. They were considered by Brenner (1963) to be of Barremian age, while Doyle (1992) indicated an early Aptian age for the lower part of pollen Zone I in the Potomac Group sequence.

The fossils were extracted from the sediment samples by sieving in water. Adhering mineral matrix was removed by treatment with hydrofluoric acid (40 %) and hydrochloric acid (10 %), followed by rinsing in water, following standard methods (Friis *et al.*, 1988). The fossils were investigated initially using a stereomicroscope. For scanning electron microscopy (SEM), specimens were mounted on cleaned aluminium stubs, coated with gold for 90 s in a sputter coater, and examined with a Hitachi field emission-scanning electron microscope at 2 kV. Selected seeds were macerated for light microscope studies, using nitric acid followed by ammonia or by using sodium hypochlorate. Specimens and preparations are housed in the Swedish

Museum of Natural History, Stockholm (S) and in the Field Museum, Chicago (PP).

## RESULTS

### Formal descriptions

#### SPERMATOPHYTA

#### Order Gnetales

#### Genus *Ephedra* L.

#### *Ephedra portugallica* sp. nov. (Figs 1A–K and 2A–F).

*Specific diagnosis.* Fossil seeds and *in situ* pollen with their general organization and structure as in modern *Ephedra* (nucellus surrounded by integument and outer sclerenchymatous envelope, long micropylar tube extending beyond the envelope, papillae on inner apical part of envelope, pollen exine shed during germination), and with the following defining features:

Seeds small, 0.85–1.2 mm long, 0.7–0.8 mm wide, ovoid with rounded base and pointed apex. Attachment scar prominent, rounded to slightly squared, approx. 0.25 mm in diameter. Sclerenchymatic cells of outer seed envelope narrowly rectangular, approx. 10–30 µm long, longitudinally arranged. Pollen approx. 33–40 µm long, 20–25 µm wide, approx. 10–15 ridges on exposed side (inferred total of 20–30 ridges).

*Etymology.* Named from Portugal, where the fossils were found.

*Holotype.* S107680 (Buarcos sample 157, Figs 1A, 2A–C).

*Paratypes.* S107678–S107679, S107681, S136808 (Buarcos sample 157); S107682, S107684–S107686, S148070–S148072, 148102 (Buarcos sample 209); S107687–S107689, S148077–S148078 (Buarcos sample 211); S107683, S134042, S148073, S148087–S148088, S148090, S148092, S148094–S148096 (Buarcos sample 243); S148060 (Buarcos sample 267).

*Locality.* Buarcos, North of Figueira da Foz, Portugal (40°09'54"N; 8°52'11"W).

*Stratigraphic position and age.* Calvaria Member, Figueira da Foz Formation. Early Cretaceous (late Aptian or early Albian).

*Description and comments on *Ephedra portugallica*.* This seed type is the most abundant and informative of the fossil ephedroid seeds from the Buarcos locality and is known from more than 50 specimens.

*Shape and size of seeds.* The seeds are small, about 0.85–1.2 mm long and 0.7–0.8 mm wide. They are often compressed to various degrees but have a basically ovoid shape with a rounded base and pointed apex (Fig. 1A–G). In cross-section they are almost circular, to faintly four-angled but they are more distinctly four-angled towards the apex (Fig. 2A). In some specimens the four-angled form is emphasized by distinct longitudinal ridges that probably mark the position of vascular bundles along the four

edges (Figs 1A, B and E). The seeds sometimes split apically into four valves along the ridges (Fig. 1G). The basal attachment scar is distinct and circular to four-angled, approx. 0.25 mm in diameter (Fig. 1B, C and F).

*Organization and structure of seeds.* The seeds are composed of three distinct parts: an innermost membranous nucellus preserved as a delicate cuticle, a membranous integument and a thick sclerenchymatic outer seed envelope. The nucellus has been observed only in macerated specimens. It is ovate to elliptic in longitudinal outline with an irregular apical area. The integument is ovate to elliptic in longitudinal outline and extended at the apex into a long, narrow micropylar tube (Fig. 2E) that is circular in cross-section (Fig. 2A). The outer seed envelope consists of an outer epidermis, a middle sclerenchyma layer, and an inner epidermis. In one specimen the outer epidermis is well preserved (Fig. 1B), but otherwise this layer is usually abraded over most of the seed surface except for small areas in the apical region. The shape and size of the epidermal cells are unclear. The sclerenchyma layer constitutes the bulk of the envelope and the sclerenchyma cells are generally well preserved. They are narrowly rectangular, about 10–30 µm long, and longitudinally arranged (Fig. 1H and I).

The inner epidermis of the seed envelope has, in the apical region adjacent to the micropylar tube, a distinct and well-preserved zone of papillae extending from the top of the seed envelope and about 0.17 mm downwards. The papillae are observed in specimens where the envelope is split apically (Fig. 1G, J and K), and sometimes in well-preserved intact specimens where the micropylar area is not filled in with debris or collapsed cells. Each papilla is about 10 µm long, but they decrease in size and prominence towards the base of the papillate zone.

*In-situ pollen grains.* Pollen grains were found *in situ* in the micropylar tube of three specimens. In two specimens (Fig. 1A and D) pollen grains were observed at the top of the micropyle of seeds mounted for SEM (Fig. 2A–D). The pollen grains are ellipsoid, approx. 33–40 µm long, and approx. 20 µm wide, with 10–15 ridges on the exposed surface; the inferred number of ridges for the whole grain is thus 20–30. There is no visible aperture. The surface of the pollen wall is indistinctly rugulate (Fig. 2C). In the macerated specimen (Fig. 2E and F) pollen grains were observed inside the integument in the micropylar tube near the apex of the nucellus. These pollen grains show upcurled pollen exines (Fig. 2F).

#### *Ephedra drewriensis* sp. nov. (Figs 3A–K and 4A–D)

*Specific diagnosis.* Fossil seeds and *in situ* pollen with their general organization and structure as in modern *Ephedra* (nucellus surrounded by integument and outer sclerenchymatous envelope, long micropylar tube extending beyond the envelope, papillae on inner apical part of envelope, pollen exine shed during germination), and with the following defining features:

Seeds small, 1.2 and 1.4 mm long, 0.8–0.9 mm wide, ellipsoid with rounded base and short pointed apex. Attachment scar indistinct, rounded, approx. 0.15 mm in diameter.



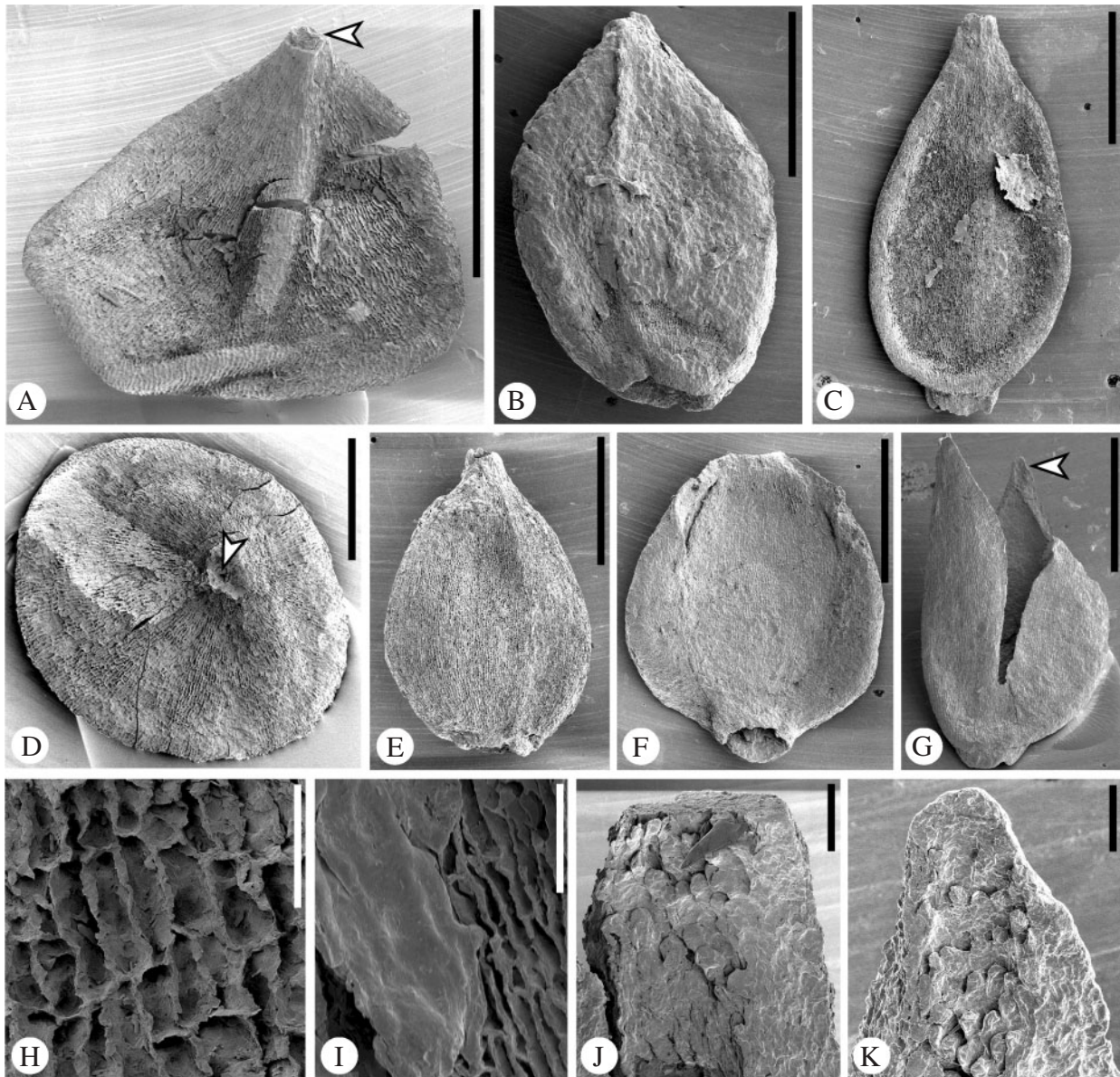


FIG. 1. *Ephedra portugallica* sp. nov., Early Cretaceous seeds from Buarcos, Portugal. (A) Holotype with *in-situ* polyplicate pollen grains indicated by an arrow (S107680). (B–F) Paratypes showing the ovoid form, the rounded base, the pointed apex and the prominent attachment scar (B, S107687; C, S107679; D, S148060; E, S107682; F, S107678). The arrow in (D) indicates an *in-situ* pollen grain. (G) The seed envelope of this specimen has split from the apex, exposing papillae on the inner apical region (S107685). (H) Sclerenchyma cells of the outer seed envelope (S148060). (I) Remains of the epidermis partly covering the sclerenchyma cells (S107687). (J, K) Papillae on the apical, inner part of the seed envelope (J, S107681; K, S107685). Scale bars: A–C = 500  $\mu$ m; D = 300  $\mu$ m; E–G = 500  $\mu$ m; H–K = 30  $\mu$ m.

Sclerenchymatic cells of outer seed envelope narrowly rectangular, up to 70  $\mu$ m long, longitudinally arranged. Pollen approx. 50  $\mu$ m long, 20  $\mu$ m wide, narrowly ribbed, six or seven ridges on exposed side (inferred total of 12–14 ridges).

**Etymology.** Named from Drewry's Bluff, where the fossils were found.

**Holotype.** PP44843 (Drewry's Bluff sample 198), (Fig. 3A and J).

**Paratypes.** PP44844–PP44845, PP44878 (Drewry's Bluff sample 198); PP44854, PP44866–PP44867 (Drewry's Bluff sample 163); PP44874–PP44875 (Drewry's Bluff sample 164).

**Locality.** Drewry's Bluff on the James River, south of Richmond, Chesterfield County, VA. USA.

**Stratigraphic position and age.** Clayballs in Patuxent Formation, Potomac Group. Pollen Zone I, Early Cretaceous (early Aptian).

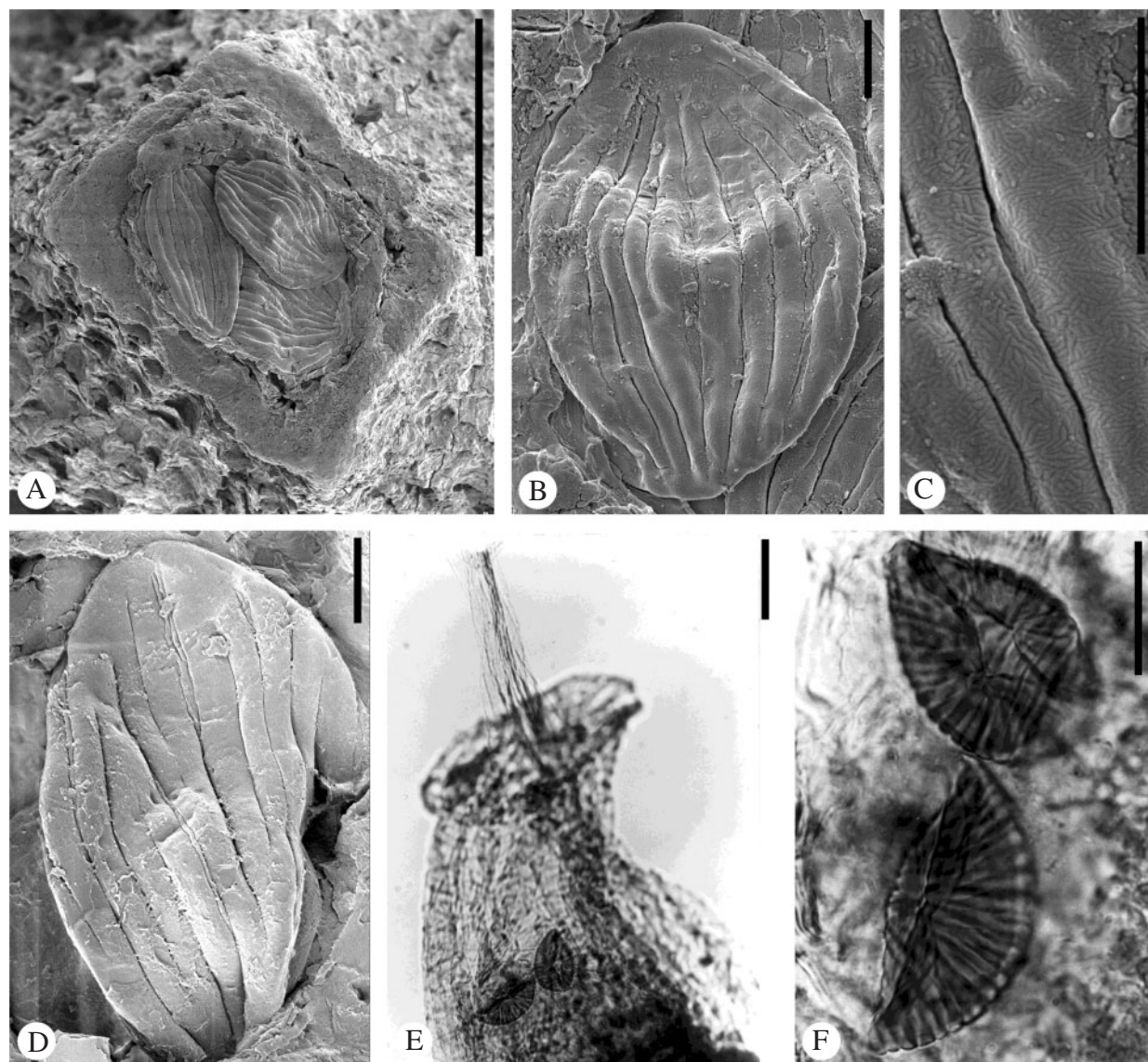


FIG. 2. *Ephedra portugallica* sp. nov. (A) apical view showing the four-angled seed envelope, the circular integument and *in-situ* polyplicate pollen (S107680); (B) *in-situ* pollen grain indicated by the arrow in Fig. 1A (S107680); (C) rugulate surface of pollen grains in the holotype (S107680); (D) *in-situ* pollen grain indicated by the arrow in Fig. 1D (S148060); (E, F) macerated seed showing extended micropylar tube, seed envelope and two discarded pollen exines inside the seed (S136808). Scale bars: A = 50  $\mu$ m; B–D = 5  $\mu$ m; E = 60  $\mu$ m; F = 20  $\mu$ m.

*Description and comments on Ephedra drewriensis.* About 10–15 seeds of this type were found in the samples from Drewry's Bluff.

*Shape and size of seeds.* The seeds are about 1.2–1.4 mm long and 0.8–0.9 mm wide. They are compressed to various degrees but have a basically ellipsoid shape with a rounded base and pointed apex. They are often flattened and faintly four-angled in cross-section, but they are more distinctly four-angled towards the apex (Fig. 3A–G). Weak longitudinal ridges correspond to the position of vascular bundles (Fig. 3B, C, E and H). The seed envelope sometimes split apically into four valves (Fig. 3G). The basal attachment scar is indistinct, circular, and approx. 0.15 mm in diameter.

*Organization and structure of seeds.* The seeds are composed of three distinct tissues: an innermost nucellus surrounded by a membranous integument, and a thick sclerenchymatic outer seed envelope. The nucellus has been observed only in macerated specimens. It is ovate to elliptic in shape and with an irregular apical area. The integument is elliptic in shape and apically extended into a micropylar tube that is circular in cross-section (Fig. 4A, B and D). The outer seed envelope consists of an outer epidermis, a middle sclerenchyma layer and an inner epidermis. The outer epidermis is partly preserved in some specimens (Fig. 3B and D), and consists of more or less isodiametric cells approx. 9–13  $\mu$ m across. The sclerenchyma cells are generally well preserved, narrowly rectangular up to 70  $\mu$ m long, and longitudinally arranged (Fig. 3H and I).



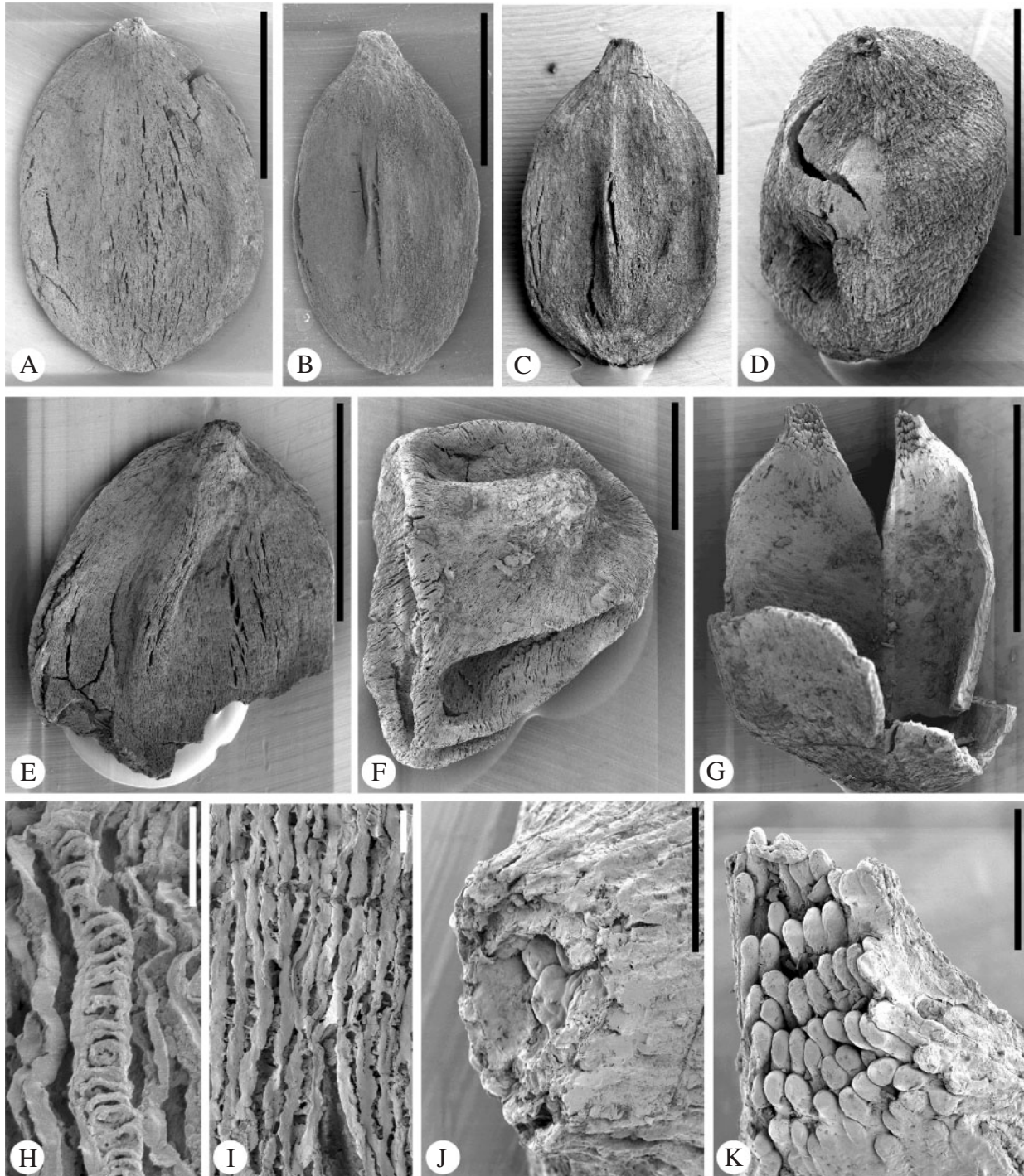


FIG. 3. *Ephedra drewriensis* sp. nov., Early Cretaceous seeds from Drewry's Bluff, Virginia, USA. (A) Holotype, showing the ellipsoid form of the seeds and papillae on the seed envelope (PP44843). (B–F) Paratypes showing the faintly four-angled seed envelope and the short, pointed apex (B, PP44854; C, PP44875; D, PP44874; E, PP44844; F, PP44878). (G) Open specimen showing exposed papillae on the apical, inner surface of the seed envelope (PP44867). (H) Xylem cells with spiral to helical wall pattern on the ridge of the seed in (C) (PP44875). (I) Sclerenchyma cells of *Ephedra drewriensis* are narrowly rectangular, up to 70  $\mu\text{m}$  long and longitudinally arranged (PP44844). (J) Papillae on the seed envelopes of an intact seed (PP44843). (K) Papillae on an open seed envelope (PP44867). Scale bars: A–E = 600  $\mu\text{m}$ ; F = 300  $\mu\text{m}$ ; G = 600  $\mu\text{m}$ ; H and I = 10  $\mu\text{m}$ ; J and K = 60  $\mu\text{m}$ .

Papillae occur on the inner surface of the outer seed envelope in an area extending from the apex to approx. 0.17 mm downwards. The papillae are 10  $\mu\text{m}$  wide and 10–20  $\mu\text{m}$  long, but the lowermost papillae are less

well developed. Papillae can be observed in split open seed envelopes (Fig. 3G and K), in some intact seeds (Fig. 3A, F and J) and in macerated specimens (Fig. 4D).

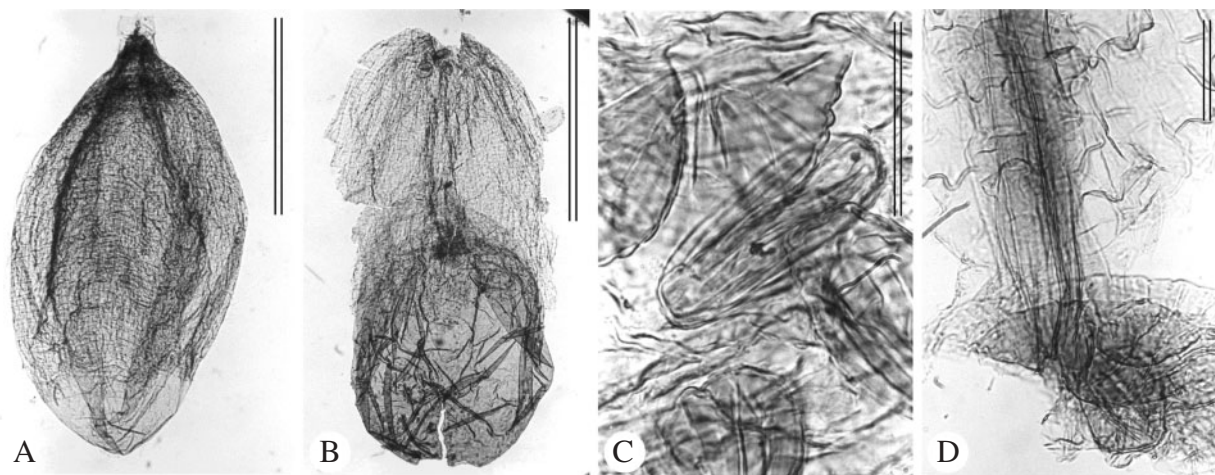


FIG. 4. Macerated seed of *Ephedra drewriensis* sp. nov. (PP44875): (A) seed envelope and integument; (B) seed envelope, integument and nucellus, *in-situ* pollen in the upper part; (C) close up of polyplicate pollen grains with a non-germinated grain in the middle surrounded by discarded exines; (D) micropylar tube and seed envelope with papillae. Scale bars: A and B = 600 µm; C = 30 µm; D = 45 µm.

**In-situ pollen grains.** Specimen PP44875 (Figs 3C and 4A–D) was macerated and at least five polyplicate pollen grains were found *in situ* inside the integument (Fig. 4C). They are narrowly elliptic, nearly 50 µm long and 20 µm wide, ribbed with six or seven ridges on one side. The total number of ridges inferred for pollen grains of *Ephedra drewriensis* is 12–14. There is no visible aperture in the pollen grains. Some grains are intact, but there are also several upcurled pollen exines (Fig. 4C).

***Ephedrispermum* gen. nov.** (Fig. 5A–N)

**Generic diagnosis.** Fossil seeds with their general organization and structure similar to modern *Ephedra*. Seeds ovoid to almost spherical with a short pointed apex. Nucellus and integument surrounded by an outer sclerenchymatous seed envelope, seed envelope distinctly four-angled towards apex. Sclerenchyma cells rectangular and in two layers, outer layer cells transversely arranged, inner layer longitudinally arranged. Papillae on seed envelope not observed. Pollen grains narrowly ribbed.

**Etymology.** These *Ephedra*-like seeds have been named from *Ephedra* and *spermum* meaning seed.

**Type species.** *Ephedrispermum lusitanicum* gen. et sp. nov.

**Comments on the new genus *Ephedrispermum*.** The seeds are similar in organization and structure to seeds of *Ephedra*, but discarded pollen exines and papillae on the seed envelope, characters that are diagnostic of extant *Ephedra*, have not been detected. Seeds of *Ephedrispermum* further differ from *Ephedra* in the cross-wise pattern of sclerenchyma cells on the seed envelope. As far as is known, this pattern is not present in *Ephedra* and, therefore, a new genus within the Gnetales has been established for these seeds.

***Ephedrispermum lusitanicum* gen. et sp. nov.** (Fig. 5A–N)

**Specific diagnosis.** As for the genus with the following additions. Seeds minute, 0.6–0.9 mm long, 0.6–0.8 mm

wide, ovoid to spherical with short pointed apex. Attachment scar prominent, rounded, approx. 0.1 mm in diameter. Outer layer of sclerenchyma cells rectangular, up to 30 µm long, transversely arranged. Inner layer cells up to 30 µm long, longitudinally arranged. Pollen grains approx. 23 µm long, 10 µm wide, approx. six ridges on the exposed side (inferred total of up to 12 ridges).

**Etymology.** From Lusitania, name of Roman province covering central Portugal where the fossils were collected.

**Holotype.** S148062 (Buarcos sample 244; Fig. 5A and L).

**Paratypes.** S148062–S148064 (Buarcos sample 244); S148079–S148083 (Buarcos sample 157); S148097, S148099 (Buarcos sample 209); S148076 (Buarcos sample 211); S148074, S148085–S148086, S148091 (Buarcos sample 243); and S148106–S148109, S148111–S148112, S148114–S148120, S148125, S149286–S149289 (Torres Vedras sample 43).

**Locality.** Buarcos, North of Figueira da Foz, Portugal (40°09'54"N; 8°52'11"W).

**Stratigraphic position.** Calvaria Member, Figueira da Foz Formation. Early Cretaceous (late Aptian or early Albian).

**Description and comments on *Ephedrispermum lusitanicum*.** About 35 seeds of this type have been found in samples from Torres Vedras and Buarcos. They are typically less well preserved than seeds of *Ephedra portugallica* and *Ephedra drewriensis*. This is particularly true for seeds from the Buarcos locality.

**Shape and size of seeds.** Seeds are minute, 0.6–0.9 mm long and 0.6–0.8 mm wide, broadly ovoid to almost spherical in shape (Fig. 5A–K) and faintly four-angled in cross-section except at the apex where they are distinctly four-angled (Fig. 5A–K). The seeds are rounded at the base and have a circular attachment scar, about 0.1 mm in diameter, and a short, pointed apex (Fig. 5E and F).

**Organization and structure of seeds.** The seeds are apparently identical to other ephedroid seeds from Buarcos



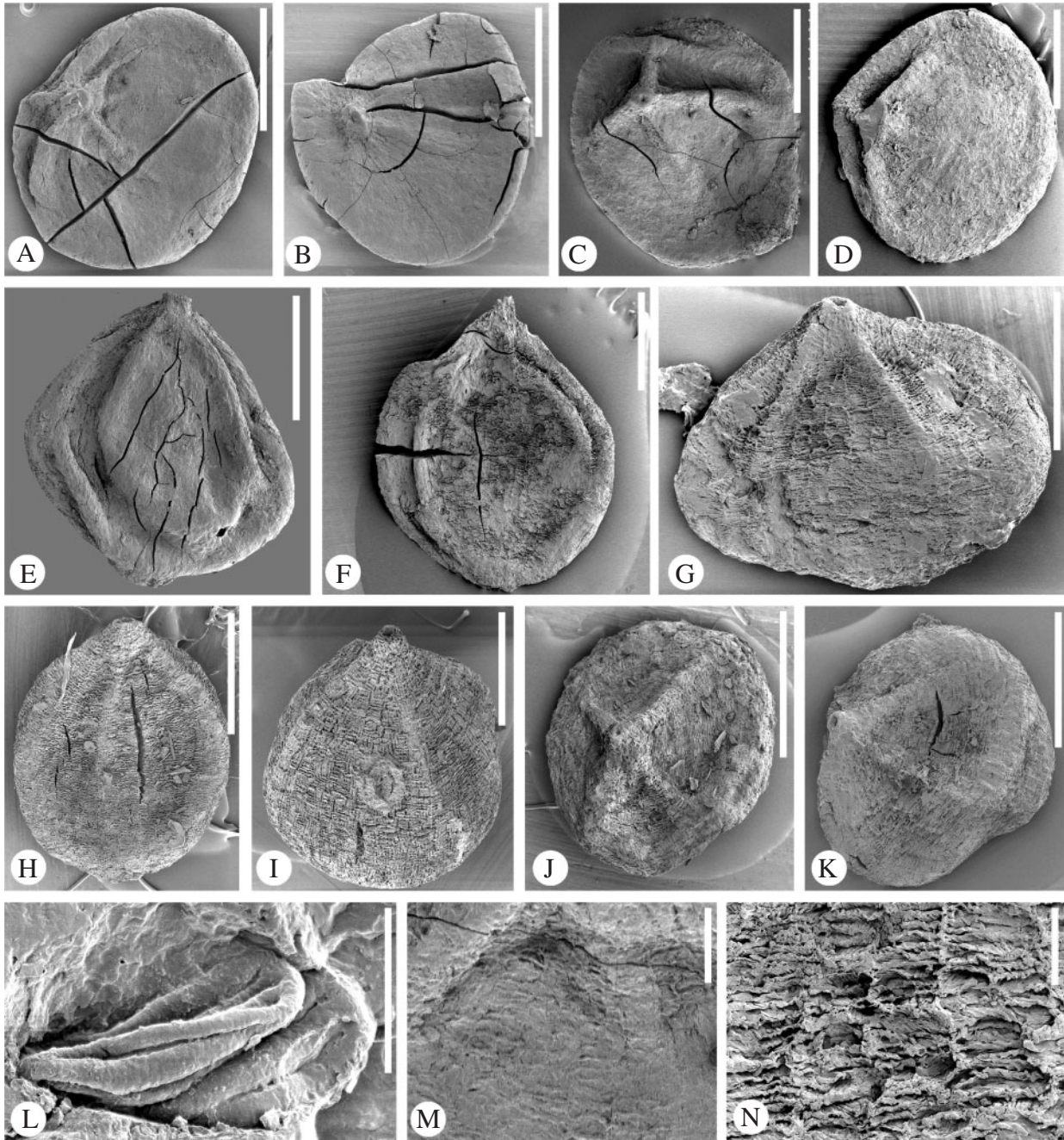


FIG. 5. *Ephedrispermum lusitanicum* gen. et sp. nov., Early Cretaceous seeds from Buarcos and Torres Vedras, Portugal. (A) Holotype: *in-situ* pollen grains in the micropyle (S148062). (B–K) Paratypes showing the rounded shape of the seed envelope, with a four-angled, pointed apex. A layer of longitudinally arranged sclerenchyma cells is overlain by a layer of transversely arranged cells (B, S148064; C, S148074; D, S148086; E, S148099; F, S148081; G, S148091; H, S148116; I, S149286; J, S148115; K, S148120). (L) *In-situ* polyplicate pollen grains in the holotype specimen (S148062). (M) The outer layer of transversely arranged sclerenchyma in the seed envelope is visible but poorly preserved in the holotype (S148062). (N) Well-preserved sclerenchyma cells (close up of G) (S148091). Scale bars: A–K = 300 µm; L = 12 µm; M and N = 30 µm.

in organization and structure, although they are smaller, nearly spherical and not as well preserved. Maceration of seeds failed to provide information on the nucellus, the shape of micropylar tube, and the inner epidermis of the outer seed envelope. The outer epidermis of the outer seed envelope is poorly preserved in most specimens. The sclerenchymatic part of the seed envelope

consists of at least two cell layers. Cells in the outer layer are rectangular, up to 30 µm long and arranged in transverse rows (Fig. 5 M and N). Cells of the inner layer are more narrowly rectangular, up to 30 µm long and longitudinally aligned. The micropylar region is generally poorly preserved and papillae have not been observed.



TABLE 1. Morphological variation in seed and pollen characters of *Erdtmanithecales*, *Ephedrispermum* and *Ephedra*

	<i>Erdtmanithecales</i>	<i>Ephedrispermum lusitanicum</i>	<i>Ephedra portugallica</i>	<i>Ephedra drewriensis</i>	Extant <i>Ephedra</i> *
Length/width of pollen grains	approx. 15–20 µm/ 11–12 µm	approx. 23 µm/ 10 µm	approx. 33–40 µm/ 20–25 µm	approx. 50 µm/ 20 µm	(25–)30–70(–80) µm/ (16–)20–40(–50) µm
Inferred total no. of ridges on pollen grains <sup>†</sup>	3	<12 (?)	20–30	12–14	Variable, 4–20
Discarded pollen exines found <i>in situ</i>	No	No	Yes	Yes	Yes <sup>‡</sup>
Seed length (mm)	1.0–1.2	0.6–0.9	0.8–1.2	1.2–1.4	6–7 or more
No. of angles on seed envelope	3	4	4	4	2-, 3-, 4-angled or globulose
Seed form	Ovoid	Spherical	Ovoid	Ellipsoid	Variable <sup>§</sup>
Papillae on seed envelope	No	No (?)	Yes	Yes	Yes
Orientation of outermost sclerenchymatic cells	Isodiametric	Transverse	Longitudinal	Longitudinal	Longitudinal
Length of sclerenchymatic cells	10 µm	c. 30 µm	10–30 µm	Up to 70 µm	Variable <sup>¶</sup> , 50–200 µm

\* Note that we do not cover the morphological variation of all extant species here.

<sup>†</sup> Generally, the total number of ridges cannot be observed in fossil grains, but is inferred from the number of visible ridges on the exposed side of the grain.

<sup>‡</sup> See El-Ghazaly *et al.* (1998).

<sup>§</sup> Often ovoid, but sometimes ellipsoid or globulose.

<sup>¶</sup> Nine species from all the major clades were investigated: *Ephedra alata* C-311 (S), *E. altissima* C-635 (S), *E. antisiphilitica* C-610 (S), *E. californica* C-598 (S), *E. distachya* C-535 (S), *E. equisetina* C-461 (S), *E. frustillata* 03–1721 (S), *E. intermedia* C-326 (S), *E. likiangensis* 03–926 (S).

*In-situ* pollen grains. Two specimens (Fig. 5A and B) have pollen grains in the micropyle. The pollen grains are polyplicate and small, only approx. 23 µm long and 10 µm wide. The grains in the holotype (Fig. 5A and L) have up to six exposed ridges (the total number of ridges is probably less than 12). The poorly preserved pollen grain in the micropyle of the paratype S148064 (Fig. 5B, grain not shown in close up) is only 15 µm long and has two or three ridges. This is likely to be a foreign grain.

## DISCUSSION

### Systematic and nomenclatural comments

The fossil seeds described here share many characters with each other and with seeds of extant *Ephedra*. These include a sclerenchymatous outer seed envelope that surrounds the nucellus and integument, a long micropylar tube that extends beyond the outer envelope, and *in situ* polyplicate pollen grains. Based on differences in pollen features, in the shape and size of the seeds and in the cellular details of the seed wall (Table 1), they have been described as three new species. Two of the species have papillae on the inner, apical part of the seed envelope and pollen that shed the exine during germination. This character combination is only known for seeds of *Ephedra* and they have been assigned to two fossil species of the extant genus: *Ephedra portugallica* and *Ephedra drewriensis*.

The third species also has general characters of *Ephedra* including a sclerenchymatic seed envelope and polyplicate pollen *in situ* in the micropyle. However, two diagnostic characters of *Ephedra* (discarded pollen exines and papillae on the seed envelope) have not been observed in any specimen of this species. Further, these seeds differ from *Ephedra* in the cross-wise pattern of sclerenchyma cells on the seed envelope, a pattern that as far as is known is not

present in *Ephedra* and, therefore, a new genus has been established, *Ephedrispermum*, to accommodate these seeds.

### Interpretations of the new fossils and comparison with extant species

*Seed characters.* Seeds of the three new fossil species are all small, ranging in length between 0.6 and 1.6 mm, and all possess a slightly four-angled sclerenchymatic outer seed envelope with a rounded base and a pointed apex (Figs 1–5). The shape of the fossil seeds varies from ovoid in *Ephedra portugallica*, ellipsoid in *Ephedra drewriensis* to more globose in *Ephedrispermum lusitanicum*. Many extant species have ovoid seeds, but more ellipsoid and globose seeds also occur. The apex can be distinctly or less prominently pointed, in the fossils as well as in extant seeds. In the fossils, four longitudinal ridges extend from the apex and the seed envelope is sometimes split from the apex into four valves. Xylem cells with a spiral to helical wall pattern have been observed on the longitudinal ridge of one fossil (Figs 3C and H) and the ridges probably correspond to the position of vascular bundles. The four-angled nature is evident in all the fossils, whereas the shape of extant seeds varies from two to three to four angles (four-angled seed of *Ephedra californica* shown in Fig. 6A). The shape (the number of angles) of extant seeds is partly related to the number of seeds in the cone, a character that varies among and within species.

The outer seed envelope in *Ephedra* seeds consists of an outer epidermis, a middle sclerenchyma layer, and an inner epidermis. In the fossils, the outer epidermis is typically abraded over most of the seed surface except for small areas in the apical region, but the sclerenchyma cells that comprise the bulk of the envelope are generally well preserved in all three species. In *Ephedra drewriensis* the sclerenchyma cells are very long and narrow, up to

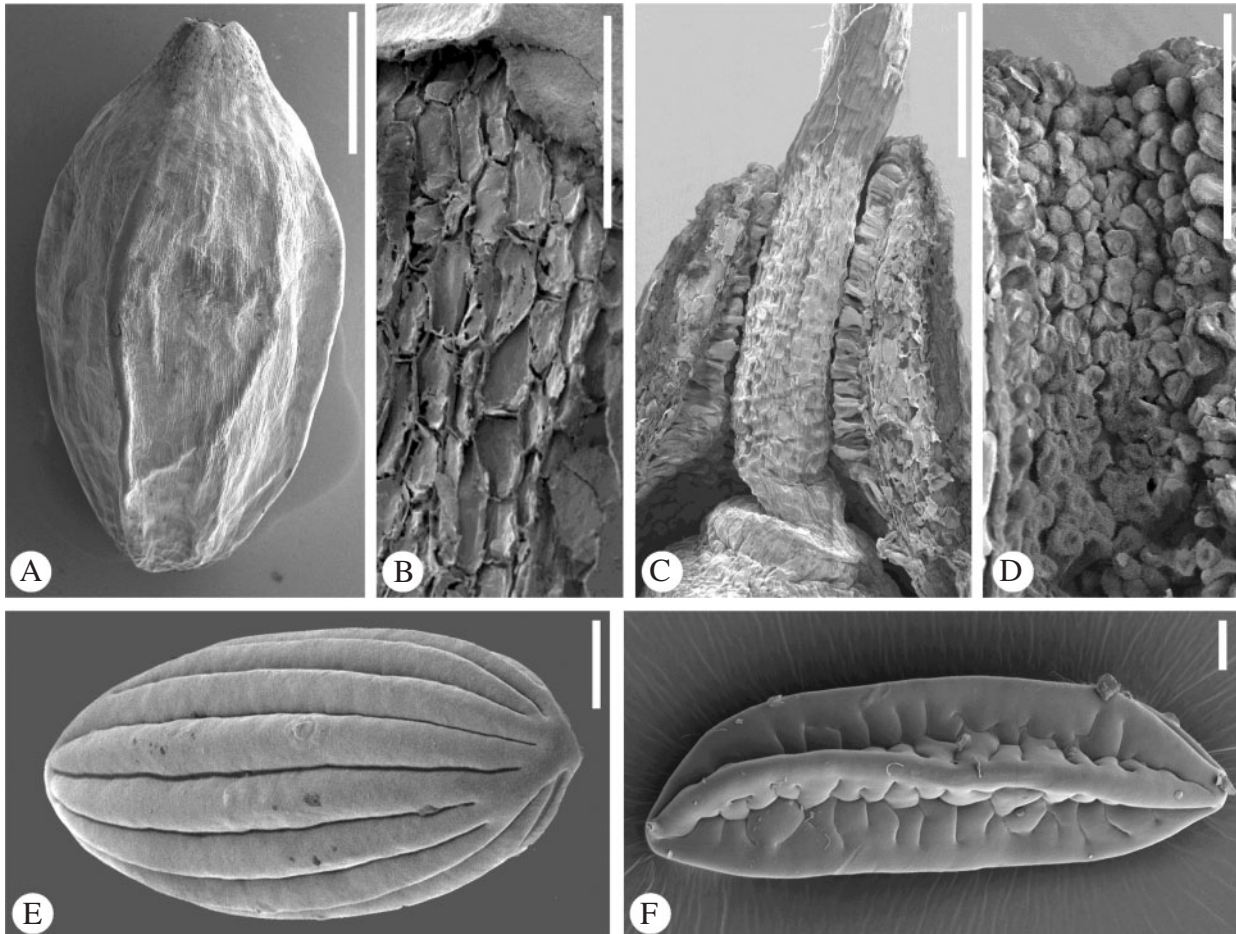


FIG. 6. Seeds and pollen of extant *Ephedra*: (A) *Ephedra californica*, seed (S-C598); (B) *Ephedra alata*, sclerenchymatic cells of the seed envelope (S-C311); (C) *Ephedra likiangensis*, micropylar tube supported by papillae on the seed envelope (S-03926); (D) *Ephedra californica*, papillae on the seed envelope (S-C598); (E) *Ephedra frustillata*, pollen grain (S-03-1728); (F) *Ephedra intermedia*, pollen grain (S-C324). Scale bars: A = 1.5 mm; B = 100  $\mu$ m; C and D = 300  $\mu$ m; E and F = 5  $\mu$ m.

70  $\mu$ m long, while those of the Portuguese species *Ephedra portugallica* and *Ephedrispermum lusitanicum* are 30  $\mu$ m or shorter. In *Ephedra portugallica* and *Ephedra drewriensis*, these cells are longitudinally arranged. The seeds of *Ephedrispermum lusitanicum* are often preserved slightly differently and the shape and size of the sclerenchyma cells are not always clear. Species with well-preserved cell patterns reveal an outer layer of transversely arranged sclerenchymatic cells covering an inner layer of longitudinally arranged cells, which together give the seeds a chequered appearance (Fig. 5G–J and M–N), not known from seeds of extant *Ephedra*. This pattern is less clear in some specimens (Fig. 5A–C and E), but is still obvious on parts of these seeds upon careful examination (Fig. 5M).

The shape and arrangement of sclerenchymatic cells of the seed envelope were investigated for nine extant species from all major subgroups of *Ephedra* (see Table 1). Removal of the epidermis revealed a layer of longitudinally arranged sclerenchymatic cells that range in length from approx. 50  $\mu$ m (Fig. 6B) to 200  $\mu$ m or more. These cells gradually decrease in length towards the apex. From this

limited sample it seems as if Old World species on average have shorter cells than New World species. The same pattern is observed in the fossils.

Maceration of the seeds to isolate acid resistant cuticles and pollen walls, shows both the cuticles of the outer seed envelope and the thin cuticles of the integument and nucellus (Figs 2E and 4A and B). The main body of the integument is ovate to elliptic in lateral view and tightly adheres to the nucellus. Apically it is extended into a long micropylar tube that protrudes beyond the outer seed envelope (Figs 2E and 4A and D). The protruding part of the tube breaks easily, which is obvious in the fossils as well as in extant seeds. Inside the envelope, the micropylar tube is supported by papillae (Fig. 6C and D) located on the inside of the upper part of the outer seed envelope (Thoday and Berridge, 1912; Rydin *et al.*, 2004). Occurrence of papillae on the outer seed envelope was investigated for 25 extant *Ephedra* species representing major subgroups (Rydin *et al.*, 2004); all possessed this character.

The fossils *Ephedra drewriensis* and *Ephedra portugallica* also have papillae, which may be observed in specimens where the envelope is split apically (Figs 1G, J and K



and 3G and K), in some well-preserved intact specimens (e.g. Fig. 3J), and also in macerated specimens (Fig. 4D). In extant and fossil *Ephedra*, the papillae adhere to the micropylar tube (Fig. 6C) and are gradually transformed downwards into ordinary cells of the inner epidermis of the envelope (Fig. 3G and K). The papillae of the fossil seeds are identical in shape and position to those of extant *Ephedra*. Papillae were not found in *Ephedrispermum lusitanicum*, but no seed of this type had split open prior to preservation, and papillae can be difficult to see in intact seeds.

**Pollen characters.** Polyplicate pollen grains (Fig. 6E and F) occur in all extant species of *Ephedra*, and have also been found *in situ* inside the fossil seeds. The surface of the pollen wall is indistinctly rugulate in the holotype of *Ephedra portugallica* (Fig. 2C), but this feature is not seen in other specimens and it is unclear if this is a result of the fossilization process. The pollen grains are narrowly ribbed and about 33–40 µm long in *Ephedra portugallica*, about 50 µm long in *Ephedra drewriensis*, and only about 23 µm long in *Ephedrispermum lusitanicum*. Pollen of extant *Ephedra* ranges between 30 and 70 µm in length, 40–50 µm being common in many species. The pollen grains of *Ephedrispermum lusitanicum* are thus smaller than what is usually seen in extant taxa.

The number of ridges on pollen grains of extant *Ephedra* differs among species. According to Kubitzki (1990), the range is from 6 to 18, but Steeves and Barghoorn (1959) report up to 20 ridges for *Ephedra alata*. Intraspecific variation has been reported for grains of extant *Ephedra* (Steeves and Barghoorn, 1959; El-Ghazaly and Rowley, 1997; Ickert-Bond *et al.*, 2003). In the fossils, pollen grains are always preserved inside the micropyles and are often folded or overlapping. It is not possible to get a three-dimensional view of these grains that exposes all sides, and it is therefore difficult to assess the exact number of ridges. Therefore, the number of visible ridges (generally the number of ridges on one side) and the total number of ridges (inferred to be about twice as many) are reported. Grains of *Ephedra portugallica* have 10–15 exposed ridges (inferred total of 20–30) (Figs 2A, B and D). Pollen grains of *Ephedra drewriensis* have fewer ridges, only six or seven on the exposed side (inferred total of 12–14) (Fig. 4C). The numbers of ridges on the fossil pollen grains of *Ephedra drewriensis* are well within the range of variation seen in modern species, whereas the inferred total of 20–30 ridges in *Ephedra portugallica* (Fig. 2A, B and D) is higher than what is generally seen in extant taxa. In *Ephedrispermum lusitanicum*, one of the grains in the holotype has six visible ridges (total number probably <12) (Fig. 5L). The other grain conceivably has fewer ridges, but it is partly covered and the number of ridges is difficult to assess. A single pollen grain observed in paratype S148064 (Fig. 5B, not shown in close up), is only 15 µm and seems to have two or three ridges. This grain is covered in a gluey substance and is considered to be foreign. Thus, the variation in size and number of ridges seen in grains of *Ephedrispermum lusitanicum* is probably an artefact owing to one partly covered and one foreign grain.

The exine of extant *Ephedra* pollen grains splits up during germination and is shed, leaving the male gametophyte naked in a characteristic way (El-Ghazaly *et al.*, 1998). Pollen grains were found inside the micropyles at the top of the megasporangium in specimens of *Ephedra drewriensis* and in *Ephedra portugallica*. In both species these included grains with curled up, shed exines (Figs 2E and F and 4B and C), which completely match the shed, upcurled, transversely striated exines described for extant *Ephedra* (El-Ghazaly *et al.*, 1998). Pollen grains of *Welwitschia* are similar to those of *Ephedra* in size and shape but *Welwitschia* grains possess a sulcus through which germination occurs and the exine remains on the gametophyte (Rydin and Friis, 2005). The unusual habit of shedding the pollen exine prior to male gametophyte growth and pollen tube formation is evidently unique to *Ephedra* among the polyplicate pollen-producing genera of the Gnetales (Rydin and Friis, 2005).

#### Comparison with other fossil seeds and pollen

**Fossil Gnetales.** The fossils described here are currently the only coalified and three-dimensionally preserved ephedroid seeds reported from the fossil record. In addition to the three newly described species, a diversity of other *Ephedra*-like seeds has been found in samples of Early Cretaceous age from Portugal and the Potomac Group sequences (Fig. 7A–L). However, these seeds differ from those described here in key details and lack diagnostic features such as *in situ* pollen grains and papillae on the seed envelope. This material requires further study to establish their exact systematic position, but they provide additional evidence that a diversity of ephedroid species were present in the Early Cretaceous vegetation.

The only other *Ephedra* fossil in which details of seeds are preserved is *Ephedra archaeorhytidosperra*, from the Early Cretaceous Yixian Formation of north-eastern China (Yang *et al.*, 2005). It is a compression fossil of a fertile shoot that has strong, vegetative similarities to *Ephedra*. Female cones are terminal on the branches. Seeds are partly enclosed by remnants of bracts, and there are weak impressions of an extended micropylar tube. The seeds of *Ephedra archaeorhytidosperra* resemble the fossils presented here in their size, ovoid shape with a pointed apex and the presence of an extended micropyle, but are clearly different in having a prominent, longitudinal, dorsal ridge and a seed surface characterized by wavy transverse ridges.

A variety of dispersed polyplicate pollen types have previously been recorded from the Drewry's Bluff locality (Brenner, 1963). Many are clearly ephedroid but others are more similar to *Welwitschia*, e.g. those found in association with *Drewria potomacensis* (Crane and Upchurch, 1987). The pollen grains associated with *Drewria* are clearly distinct from those found *in situ* in *Ephedra drewriensis*. Grains of *Ephedra drewriensis* appear inaperturate as in pollen of extant *Ephedra*, and they shed the pollen exine during germination.

In addition, there are dispersed and *in situ* grains of *Eucommiidites* (Erdtmanithecales) at the same locality (further discussed below).

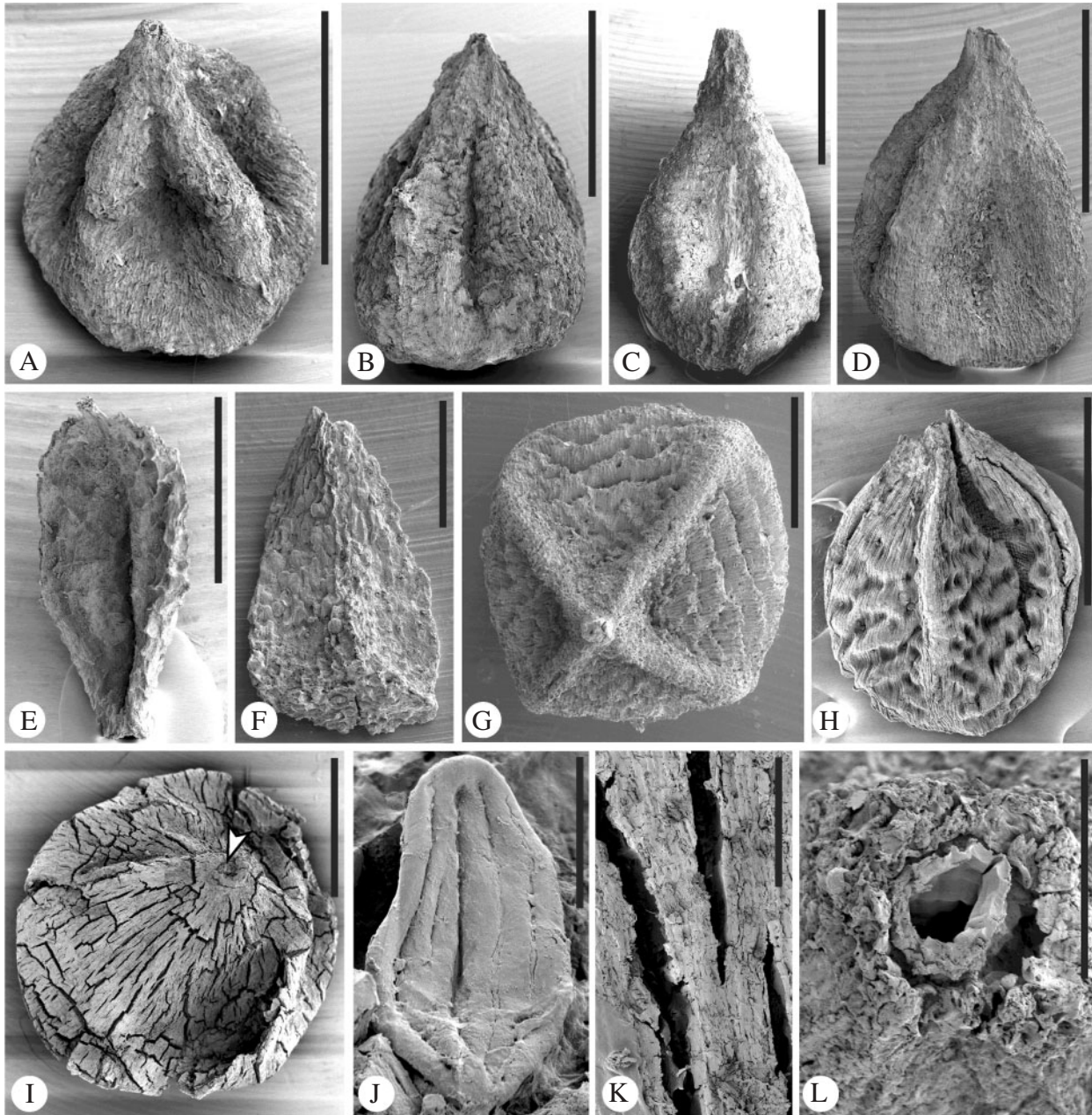


FIG. 7. Additional diversity of Early Cretaceous putatively ephedroid seeds. (A–D) Ovoid and prominently four-angled seeds, with folded seed envelope from Drewry's Bluff (A, PP44869; B, PP44848; C, PP44868; D, PP44870). (E) Obovoid seed with transverse ridges and a narrow and distinctly pointed micropylar region; Drewry's Bluff (PP44852). (F) Flattened and perhaps three-angled seed of unknown affinity; Drewry's Bluff (PP44855). (G, H) Seeds with transverse ridges; Drewry's Bluff (G, PP44857; H, PP44877). (I–K) A single seed, much larger than the other fossil seeds. The micropyle contains a polylicate pollen grain; Torres Vedras (S148121). (L) Close-up of (A) showing the circular micropylar tube surrounded by the seed envelope (PP44869). Scale bars: A–E = 600 µm; F and G = 300 µm; H and I = 600 µm; J = 12 µm; K and L = 60 µm.

*Fossil Erdtmanithecales.* Among seeds of other gymnosperms only those of the extinct Erdtmanithecales (Fig. 8A–D) are comparable in gross morphology to the fossil *Ephedra* seeds described here. Erdtmanithecales is an order of seed plants established by Friis and Pedersen (1996) to encompass dispersed pollen organs and seeds with *Eucommiidites* pollen *in situ* in the pollen sacs and inside the micropyles of the seeds.

The pollen genus *Eucommiidites* (Fig. 8D) was established by Erdtman (1948) for pollen grains from the Early Jurassic of Scania, Sweden, characterized by three elongated slits. Erdtman described the pollen as tricolpate and compared it with pollen of the angiosperm genus *Eucommia*. It was later shown that the colpi are unevenly distributed and the grains were re-interpreted as non-angiospermous pollen grains with one distal colpus and



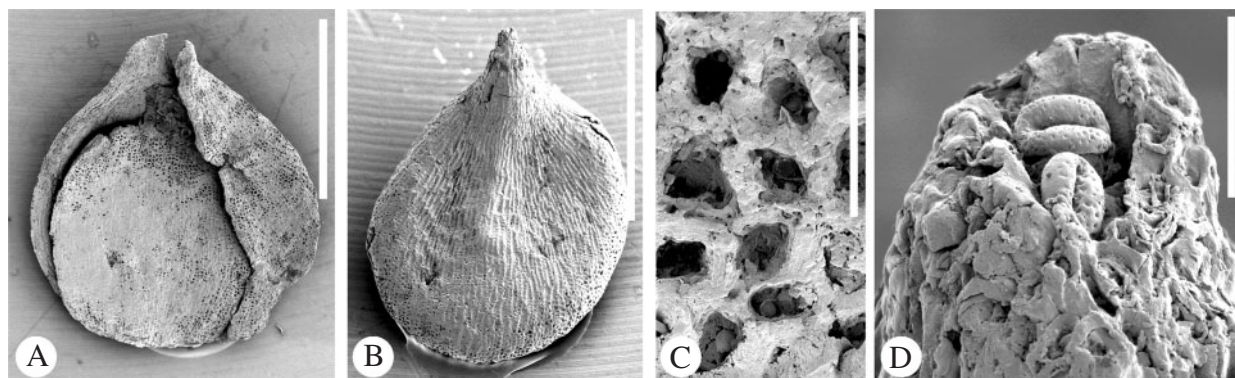


FIG. 8. Erdtmanithecales from the earliest Cretaceous of Bornholm, Denmark (*Erdtmanispermum* seeds and *Eucommiidites* pollen). (A, B) Seeds showing the three-angled nature of the seed envelope and the pointed apex (A, S148043; B, S148050). (C) Isodiametric sclerenchyma cells of the seed envelope (S148043). (D) *Eucommiidites*-type pollen with punctate surface in the micropyle of specimen in (B) (S148050). Scale bars: A and B = 600  $\mu$ m; C and D = 30  $\mu$ m.

two subsidiary, lateral colpi (Couper, 1956, 1958). A non-angiospermous affinity of the grains was later demonstrated by the discovery of pollen grains *in situ* in the micropyle of small seeds (Hughes, 1961; Brenner, 1963, 1967) (Fig. 8D).

Dispersed *Eucommiidites* pollen grains are widespread in Mesozoic palynofloras from the northern hemisphere, ranging in age from the Early Jurassic to the early Late Cretaceous, but all pollen organs and seeds discovered so far are from the Cretaceous. Pollen organs that can be assigned to the order include *Erdtmanitheca* (Pedersen *et al.*, 1989), *Eucommiitheca* (Friis and Pedersen, 1996) and *Bayeritheca* (Kvacek and Pacltová, 2001). Seeds of Erdtmanithecales include fossils assigned to *Erdtmanispermum* (Pedersen *et al.*, 1989) as well as two unnamed fossils (Hughes, 1961; Brenner, 1963, 1967). *Erdtmanitheca* is from the early Late Cretaceous (early Cenomanian) of North America (Texas), while all the other taxa are from the Early Cretaceous.

Seeds of *Erdtmanispermum balticum* (Erdtmanithecales) are coalified and comparable in preservation to the fossil *Ephedra* seeds described here. They were described from the earliest Cretaceous (Berriasian-Valanginian) sediments of Bornholm, Denmark (Pedersen *et al.*, 1989) (see also Fig. 8A–D). They are ovoid in outline with a rounded base and a long, distinctly pointed micropyle (Fig. 8A and B). The seed wall is composed of a membranous integument and an outer sclerenchymatic seed envelope. The sclerenchyma cells are small and isodiametric (approx. 10  $\mu$ m; Fig. 8C). The envelope is three-parted and gives the seed a weakly triangular cross-section. The envelope sometimes splits from the apex into three valves. In apical view the micropylar tube is circular and surrounded by the triangular envelope. Pollen grains of *Eucommiidites*-type were found inside the seeds by maceration (Pedersen *et al.*, 1989) and were also observed by SEM in the top of the micropylar tube (Fig. 8D). The two unnamed seed types with *Eucommiidites* pollen *in situ* are preserved only by their seed cuticles and the nature of the seed wall is unknown, but the specimen from Drewry's Bluff figured by Brenner (1963) clearly shows a triangular form.

Seeds of *Erdtmanispermum balticum* strongly resemble the seeds described here, in size, in their ovoid shape with a prominently pointed apical region, in the angled envelope, probably derived from bracts, and in having a long micropylar tube. *Erdtmanispermum* (Erdtmanithecales) differs from fossil *Ephedra* seeds in the three-angled rather than four-angled nature of the envelope, the absence of papillae on the envelope, and in the much smaller, isodiametric sclerenchymatic cells under the epidermis. They also differ in having a distinct megaspore membrane, which is unknown for *Ephedra*. *Eucommiidites* pollen (Erdtmanithecales) is smaller than *Ephedra* grains and distinct with only three ridges.

The microsporangiate units of *Erdtmanitheca* are described from the Early Cretaceous Vila Verde locality (Pedersen *et al.*, 1989), not far from Buarcos, in sediments that may be of approximately the same age as the Buarcos locality, and plants of Gnetales and Erdtmanithecales occur together, e.g. in the clayballs at the Drewry's Bluff locality. The possibility that small angular seeds from Buarcos and Drewry's Bluff could belong to Erdtmanithecales cannot be excluded. However, seeds of *Ephedra portugallica* and *Ephedra drewriensis* have clear affinity to *Ephedra* based on their relatively large pollen grains with many ridges and the presence of discarded exines in the micropyle. *Ephedrispermum lusitanicum* is more problematic as it has small pollen grains with few ridges. *Ephedrispermum* is, however, clearly distinct from Erdtmanithecales in the four-angled nature of the seeds, the shape of the sclerenchyma cells and in the presence of pollen grains with more than three ridges.

#### The evolutionary history of *Ephedra*

**Fossil evidence.** Polyplicate pollen grains that may resemble those of extant *Ephedra* and *Welwitschia* are common in the fossil record and extend back to the Permian (Wilson, 1962). They become more common in the Triassic (Traverse, 1988), decline again in the Jurassic and expand rapidly in both diversity and abundance in the Early Cretaceous (Crane and Lidgard, 1989; Osborn

et al., 1993; Crane, 1996). Many of the Cretaceous dispersed polyplicate pollen types most likely represent the Gnetales (e.g. Osborn et al., 1993), but the systematic position of pre-Cretaceous polyplicate pollen is more uncertain. Some of the Permian pollen grains, such as *Vittatina*, are known to be produced by extinct seed plants (peltasperms), while others are of unknown origin (Meyen, 1987).

The earliest megafossil possibly representing the Gnetales, is the Permian cone *Palaeognetaleana auspicia* that has *in situ* polyplicate pollen (Wang, 2004), but details of the organization and structure of this plant are not fully established. Pre-Cretaceous megafossils associated with the Gnetales are often morphologically distinct from extant groups and difficult to interpret. The Late Triassic plant *Dechellyia gormanii* (Ash, 1972) and the early Jurassic *Piroconites kuespertii* (van Konijnenburg-van Cittert, 1992) are other examples of fossil plants that have been compared with Gnetales but are of uncertain relationship.

A study of Cretaceous floristic diversity based on palynological assemblages suggest that there was a striking increase in gnetalean diversity simultaneously with the initial angiosperm radiation in the Early Cretaceous, but that the Gnetales underwent a significant decline again through the Late Cretaceous (Crane and Lidgard, 1989). Unequivocal gnetalean megafossils are now known from Early Cretaceous strata. The Crato flora from Brazil (Mohr and Friis, 2000; Mohr and Eklund, 2003; Mohr and Bernardes-de-Oliveira, 2004; Mohr et al., 2004; Dilcher et al., 2005) contains several ephedroid plants. The Yixian Formation in western Liaoning China, also famous for its feathered dinosaurs and other exquisitely preserved vertebrates, has proven particularly rich in gnetalean fossils. The lower part of the formation has been radiometrically dated to approx. 125 Myr, corresponding to the Barremian (Early Cretaceous), and the fossil-bearing sediments extend into the Aptian (for a review of the geology and biology of the Yixian Formation, see Zhou et al., 2003).

Several *Ephedra*-like impression fossils have been discovered from the Yixian Formation including currently unpublished material (C. Rydin, S. Q. Wu and E. M. Friis in progress). *Eragrosites changii* and *Liaoxia chenii* (Cao et al., 1998) from the Yixian Formation both have opposite branching and small cone-like reproductive structures. They were originally described as angiosperms but are clearly ephedroid and *Liaoxia* and *Eragrosites* were later combined and re-described jointly as *Ephedrites chenii* (Guo and Wu, 2000). *Ephedra archaerhytidosperra* (Yang et al., 2005), also from the Yixian Formation, has seeds with transverse laminar protuberances, comparable to those of extant *Ephedra rhytidosperra*. *Ephedrites? elegans* (Sun et al., 2001) from the Early Cretaceous of Liaoning, China, *Ephedrites sinensis* and *Ephedra exhibens* (Wu et al., 1986) from the Jurassic Xiaomeigou Formation, Qinghai, China, and *Alloephedra xingxuei* (Tao and Yang, 2003) from the Early Cretaceous Dalazi Formation in Jinlin, China, also belong to the Gnetales and are probably most closely related to *Ephedra*.

The fossils presented here provide details of the reproductive parts of Cretaceous *Ephedra*-like plants that have not been available in previously described material. They document that plants with characters such as apical papillae on the outer sclerenchymatic envelope, and polyplicate pollen grains that discard the exine during germination were already established in the Early Cretaceous. Such plants were widespread across Laurasia and were present in what is now the Old World, as well as in the New World. Together with other material that is not preserved at the same level of detail, these fossils suggest that a diversity of *Ephedra* and *Ephedra*-like plants were present in the Early Cretaceous. The only difference in general appearance between the fossil seeds and those of extant *Ephedra* is size. Seeds of modern *Ephedra* are much larger than their Cretaceous relatives. Whereas the fossils are generally <1.5 mm long, the length of extant *Ephedra* seeds is often up to 6–7 mm or more. Increase in seed size through time has been observed also for several angiosperm taxa, e.g. *Spirematospermum* (extinct Zingiberales) (Friis, 1988) and *Stratiotes* (Hydrocharitales) (Mai, 1985).

**Phylogenetic implications.** The information currently available on Cretaceous ephedroids is insufficient for detailed phylogenetic analysis, and the phylogenetically isolated nature of extant Gnetales further complicates out-group comparison. It is interesting, however, that seeds and pollen of Erdtmanithecales are very similar to those of *Ephedra*. Based mainly on the granular structure of the exine, and the possible homology between the two lateral colpi in *Eucommiidites* with the furrows of *Ephedra* and *Welwitschia* grains, it has been suggested that *Eucommiidites* may have been produced by plants related to the Gnetales (Doyle et al., 1975; Trevisan, 1980; Crane, 1985; Pedersen et al., 1989). The decussate arrangement of the microsporangiate units of *Eucommiitheca* is in agreement with the opposite, decussate or whorled phyllotaxis of the Gnetales, but the peltate microsporangia-bearing units of Erdtmanithecales are very different from those in extant Gnetales. Peltate structures are, however, present in *Aegianthus sibiricus*, supposedly gnetalean male cones from the mid-Jurassic of East Siberia (Krassilov and Bugdaeva, 1988).

Seeds of Erdtmanithecales and Gnetales are also similar in overall structure. An origin of the outer seed envelope from bracts has previously been suggested for extant Gnetales, e.g. by Crane (1985) and by Pedersen et al. (1989). This is consistent with ontogenetic studies of extant *Ephedra*, which show that the inner integument is initiated as a circular protrusion, while the seed envelope initiates as two, three or four protrusions and is apparently of foliar nature (Pearson, 1929; Takaso and Owens, 1994; Yang, 2001, 2004). The angled nature of the seed envelope is prominent in the fossil forms and it is very likely that the seed envelopes of *Ephedra*, *Ephedrispermum* (Gnetales) and *Erdtmanispermum* (Erdtmanithecales) are derived from paired or whorled bracts. It seems probable that the Cretaceous forms more evidently reflect this evolutionary origin. In conclusion, some but not all features of the Gnetales are established in the extinct plant group



TABLE 2. Further information on gene regions investigated in Rydin et al. (2004)

Sequence	No. of terminals/ in-group terminals	Total no. of characters	No. of informative characters	Variable but uninformative characters	Tree length	CI	RI
18S	45/39	1694	109	62	199	0.930	0.967
26S	48/42	1230	172	116	397	0.879	0.935
ITS	41/41	1687	170	84	391	0.703	0.873
ITS indels	41/41	17	17	0	24	0.708	0.941
<i>rcbL</i>	46/40	1356	171	50	268	0.914	0.968
<i>rps4</i>	48/42	487	100	28	143	0.979	0.993
<i>trnL-F</i>	8/8	523	1	3	—	—	—
Total evid.*	48/42	6471	739	340	1447	0.837	0.929

\* *trnL-F* region was not included in the total evidence analysis.

Erdtmanithecales, and whether this indicates a position of Erdtmanithecales as sister group to Gnetales needs to be tested as more information accumulates on Gnetales and their fossil relatives.

*Ephedrispermum lusitanicum*, described here, has rather small pollen (23 µm) and seems to lack papillae on the seed envelope, as do seeds of Erdtmanithecales, but *Ephedrispermum* is clearly distinct from Erdtmanithecales with its polyplicate pollen and four-angled seed envelope with rectangular sclerenchyma cells. The similarities between *Erdtmanispermum* (Erdtmanithecales) and *Ephedrispermum lusitanicum* (Gnetales) are probably plesiomorphies and not indications of a sister relationship between the two.

Seeds of *Ephedra portugallica*, *Ephedra drewriensis* and extant *Ephedra* are distinguished from those of *Erdtmanispermum* and *Ephedrispermum* by the presence of apical papillae on the seed envelope and pollen grains that discard the exine during germination. Resolving relationships among these fossil and extant species is difficult, because the extant genus comprise a homogenous group of species in which morphological characters are often more or less identical. Moreover, some characters are evidently homoplasious while others are sometimes not uniform within species or even within an individual (El-Ghazaly and Rowley, 1997; Huang, 2000; Ickert-Bond et al., 2003; Ickert-Bond and Wojciechowski, 2004; Rydin et al., 2004; Huang et al., 2005). Currently, the variation in anatomical details and reproductive characters among extant species of *Ephedra* is evidently complex and poorly understood [also see Ickert-Bond and Wojciechowski (2004) and Huang et al. (2005) for a discussion of extant morphology].

Evidence from molecular data provides limited information on relationships among extant species of *Ephedra*. A previous study (Rydin et al., 2004), investigated six gene regions from the chloroplast and the nuclear DNA, but all contained very few informative characters (Table 2). Most of the information came from the internal transcribed spacer (ITS) of the nuclear ribosomal DNA. As previously reported (Maggini et al., 1998), the ITS1 region of *Ephedra* is very long compared with ITS1 in angiosperms, and has three short subrepeats. However, only 170 of the total 1687 characters in ITS1 and ITS2 were informative. Phylogenetic analyses resulted in well-supported subgroups

of *Ephedra* that correspond to geographical regions (Huang, 2000; Ickert-Bond and Wojciechowski, 2004; Rydin et al., 2004; Huang et al., 2005) with African–Mediterranean species in a basal grade or clade and Asian species forming two well-supported clades (Ickert-Bond and Wojciechowski, 2004; Rydin et al., 2004). New World species are monophyletic and comprise a South American clade (Ickert-Bond and Wojciechowski, 2004; Rydin et al., 2004) and a non-monophyletic grade of North American species (Ickert-Bond and Wojciechowski, 2004). Relationships among these subgroups are not well supported, and unfortunately they are not characterized by obvious morphological synapomorphies. It is consequently difficult to assign the fossils to any particular subgroup within *Ephedra*.

*The age of Ephedra.* A recent radiation of crown group *Ephedra* (Huang and Price, 2003), might be inferred from molecular data alone, but this requires that Cretaceous *Ephedra* (e.g. the fossil plants described here) belonged to extensively distributed but now extinct stem group(s), and that the modern diversity in the genus is the result of a second radiation in the Oligocene or Miocene. However, this supposedly second radiation does not appear to be associated with any morphological novelties. The consequence of this scenario is that despite an assumed high rate of molecular evolution in the *Ephedra* stem lineage (see below), there is no obvious morphological divergence between the Cretaceous and the modern species. An analogous pattern occurs in the lycopod genus *Isoetes*. As in *Ephedra*, modern species of *Isoetes* are morphologically similar and molecular divergence is very low. But phylogenetic analysis results in a tree topology that implies an ancient speciation pattern with multiple Gondwana distributions (Rydin and Wikström, 2002). This interpretation is in line with fossil evidence (possible crown group members are documented at least from the Early Cretaceous) and indicates that, perhaps as in *Ephedra*, low molecular and morphological divergence does not necessarily mean recent speciation.

A Cretaceous origin of crown group *Ephedra* raises, however, other questions that need to be answered. An old age of *Ephedra* requires extremely low substitution rates within the crown group, because the branches are very short (see, for example, the phylograms in Rydin et al., 2002). In contrast, the branch leading to extant

*Ephedra* is very long and this has been attributed, at least in part, to a high substitution rate (Sanderson, 2002; Magallón and Sanderson, 2005). Studies have shown that the branches leading to all the extant genera of the Gnetales have the highest estimated substitution rates among seed plants (Sanderson, 2002; Magallón and Sanderson, 2005). If the crown group originated in the Early Cretaceous, there must accordingly have been a shift from a very high substitution rate in ephedran stem lineages to a very low substitution rate in the crown group.

Frequently used molecular dating methods often smooth rate variation among adjacent branches on a phylogeny (for a review of methods for estimating divergence times see, for example, Sanderson *et al.*, 2004), and the concept of temporal autocorrelation (see Gillespie, 1991) implies that substitution rate is a heritable trait that thus is likely to be similar among closely related lineages. A sudden and substantial change in this trait is of course possible, as in any trait, [remarkable differences in branch lengths among sister clades, thus differences in substitution rates, have been proposed, for instance, for the lycopods *Selaginella-Isoetes* (Rydin and Wikström, 2002; Korall and Kenrick, 2004), and filmy ferns *Hymenophyllum* s.l.-*Trichomanes* s.l. (Pryer *et al.*, 2001)], but its basis remains to be explained.

Further, currently used methods for molecular dating would not be able to manage such significant rate changes. For instance, a dramatic slowdown in rate of molecular evolution has been proposed for the eusporangiate ferns *Marattia-Angiopteris* and the tree ferns (Soltis *et al.*, 2002). These groups have comparatively short branch lengths, and despite fossil documentation at least from mid-Jurassic, probably further back, their estimated ages were substantially younger. The authors refer to *Marattiales* and tree ferns as 'molecular living fossils', consistent with their relative morphological stasis for the past 165–200 million years (Soltis *et al.*, 2002), and they argue that even dating methods that allow rate heterogeneity among lineages could not handle the dramatic decrease in rates inferred for these groups.

### Conclusions

The Early Cretaceous (late Barremian to early Albian) seeds described here are assigned to three new species, of which two have a character combination that excludes a relationship to any other group but *Ephedra*. These fossils document that key reproductive features characteristic of extant *Ephedra* (i.e. an extra-integumentary seed envelope with apical papillae, and polylicate pollen grains that shed the exine during germination), were established and widespread in the northern hemisphere in the early Aptian and have thus been retained for at least 120 million years. The third new species possesses some, but not all, of these characters. Further, a diversity of other seeds, which may belong to the ephedran lineage, have been presented briefly. The seeds have been found in sediments from what is now the Old World (Portugal) as well as in the New World (Virginia, USA) and the results demonstrate that a diversity of *Ephedra* and ephedroid species were present in the Early Cretaceous flora. The fossil seeds share some characters

with seeds of Erdtmanithecales and this supports the previously suggested relationship between Erdtmanithecales and Gnetales.

Even though these seeds document the presence of unique *Ephedra* characters in the Early Cretaceous, secure evidence on the age of *Ephedra* is not available. The homoplasious nature of many morphological characters in extant *Ephedra*, intraspecific morphological variation, and sparse information in investigated gene regions, make it difficult to resolve phylogenetic relationships within the genus and therefore to assign fossils to extant subgroups. Two alternative hypotheses on the age of *Ephedra*: an origin of extant *Ephedra* in the Oligocene-Miocene based on molecular dating analysis (Huang and Price, 2003) and a Cretaceous origin proposed here, both leave key questions unanswered. Nevertheless, because of the identical reproductive characters in fossil and extant *Ephedra* presented here, it is probable that crown group *Ephedra* is of Mesozoic origin, an idea further supported by the Early Cretaceous fossil *Ephedra archaeorhytidosperra* (Yang *et al.*, 2005), that has characters only present in some extant species.

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